Studies on the Metabolism of Human Tumors

II. Pentosenucleic Acid Synthesis in Tumor-bearing Rats*

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In the preceding paper (5) we reported attempts to evaluate tumor-host interrelationships as manifested by the synthesis of pentosenucleic acid (PNA) from labeled precursors. These studies were done with heterologous transplants, human tumors in the cheek pouch of cortisonized hamsters. At the time the precursor studies were performed, no effects attributable to the action of administered cortisone were observed. The tumors studied were a human sarcoma (Toolan's HS #1, [13]) and a human epidermoid carcinoma (Toolan's HEP #3, [13]). The precursors studied served as sources of the PNA purines of the host and the tumor. The metabolic behavior of both of these tumors, as measured by the incorporation of the labeled precursors, was essentially the same. Both tumors showed a preference for synthesis *de novo* as compared with incorporation of preformed purine moieties. There was a considerable difference in the extent to which the various purines studied were utilized by the tumors. Guanine was used very sparingly, as has been observed by others (6, 10). Hypoxanthine was used to a very limited extent, while adenine and 2,6-diaminopurine were incorporated more extensively. The latter, however, was used less extensively by the tumor than by host tissues.

The question was unanswered as to whether the tumor exhibits an incorporation pattern peculiar to that tissue or whether the incorporation observed was the host's superimposed on that of the tumor. There were several changes in the pattern of purine utilization in the host tissues which apparently resulted from the influence of the implanted tumor. Possibly these are general effects, but, on the other hand, they might be manifested only in hamsters. This problem might be clarified by the results of a similar series of experiments on the same tumors in a different host animal. In addition, there was considerable necrosis in the hamster-borne tumors. Perhaps, the results of this necrosis masked the effects of the tumor, and there is the additional possibility that observed changes in host metabolism are due to infection.

This paper presents a similar series of studies of purine metabolism in rats carrying human sarcoma implants, HS #1, which permit comparisons of the two species and of the behavior of the human tumor in each. In general, the results obtained paralleled those reported previously for hamsters carrying this tumor and HEP #3 (5). Certain lesser effects were not observed here, and it may be concluded these are not general. The fact that there are relatively few differences between the metabolic patterns observed with these two animals lends support to the premise that data obtained with human tumors in a heterologous system can permit some evaluation of the metabolism of the human tumor per se.

MATERIALS AND METHODS

Adenine-8-C^{14} (7) and guanine-8-C^{14} (2) were synthesized in these laboratories. Hypoxanthine-8-C^{14} and 2,6-diaminopurine-2-C^{14} were purchased from the Southern Research Institute, Birmingham, Alabama, and glycine-1-C^{14} from Isotopes Specialties Co., Burbank, California.

The rats used in these experiments were Wistar-strain females which had been treated with cortisone and x-ray.[1] In each experiment, six animals, average weight 60 gm., were used. The animals were given 0.1 mM/kg of the adenine, guanine, hypoxanthine, and 2,6-diaminopurine, and 0.116 mM/kg of the glycine-1-C^{14}. The precursors under study were administered intraperitoneally 10 days after implantation of the tumors. Control animals were treated identically with regard to cortisone and x-ray treatment and administration of the precursors.

The animals were sacrificed 24 hours after the administration of the precursor under study. The intestines, liver, kidneys, spleen, and tumor were removed and frozen in dry ice at once. All the samples of each tissue were pooled. The tissues were then treated as previously described (5). The sodium nucleates were isolated by salt extraction (11), the deoxypentosenucleic acid (DNA) and the PNA separated by alkaline hydrolysis (12) followed by precipitation of the DNA. The PNA fragments were hydrolyzed in acid to the free purines; the purines were precipitated as silver salts and regenerated with hydrochloric acid. The adenine and guanine were then separated

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[1] Animals were given a single 150-r total-body x-radiation prior to inoculation and four injections of cortisone (60 mg/kg) on alternate days starting with day of transplantation (13).

by paper chromatography, eluted from the paper, assayed spectrophotometrically, and prepared for counting as infinitely thin films on aluminum planchets (3). These planchets were assayed for radioactivity in an internal Geiger-Müller flow counter (Radiation Counter Laboratories, mark 12, model 1, helium-isobutane gas).

The activities have been presented as relative specific activities (RSA) where:

$$\text{RSA} = \frac{\text{counts/minute/}\mu\text{M isolated compound}}{\text{counts/minute/}\mu\text{M injected compound}} \times 100 .$$

The activities of the individual planchets were determined to within a standard error of 5 per cent, when the actual value of the RSA was 0.3 or more. All other values were 10 per cent or less, except for the glycine experiments, in which the errors were 5 per cent for RSA's of more than 0.03 (9).

Samples of the tissues from the animals studied were examined histopathologically by Dr. Stephen Sternberg of this Institute, and no evidence of abnormality was observed. The tumors contained less than 10 per cent necrotic tissue.

RESULTS

Adenine.—The incorporation of adenine into the PNA purines of the tissues of the host with and without a tumor is shown in Table 1. There is no very great difference between the tissues of the rat or between control and tumor-bearing animals as far as the synthesis of PNA adenine is concerned. The relative utilization of the administered adenine as a guanine precursor varies and, in the kidney, more guanine is formed from the adenine than in any other tissue. This same general trend was found in the hamster. The adenine is used by the tumor less extensively than by the tissues of the host.

2,6-Diaminopurine.—The utilization of 2,6-diaminopurine is shown in Table 2. The values given are for the nucleic acid guanine. The trace of activity in the adenine was too little to permit quantitation. The kidney incorporated diaminopurine into its PNA guanine to a greater extent than did any of the other tissues. This was not found in the hamster under comparable conditions. The tumor used less diaminopurine than it did adenine. The presence of the tumor has a slight effect on the use of diaminopurine for the synthesis of guanine in spleen but none on any other host tissue.

Guanine.—The incorporation of guanine into the PNA guanine of rat tissues is given in Table 3. The values are very low and consistent with previously published data (4). There has been an increase in the incorporation of the administered compound into the host tissues of the tumor-bearing animals as compared with the controls. The incorporation of the exogenously supplied guanine into the tumor PNA was negligible. No activity was detected in the samples counted, and, within the 0.05 confidence limit, this means an RSA value of 0.001.

Hypoxanthine.—The incorporation of hypoxanthine into the PNA purine is shown in Table 4. This purine serves as a precursor of both of the purines of the rat PNA but not to the same extent as was found with hamsters. It is used by the tumor, but not extensively. It is interesting to note that, when there is a tumor present, the incorporation of exogenously supplied hypoxanthine is increased in the liver, intestines, and spleen. This influence of tumor on host is reminiscent of that observed with tumor-bearing hamsters (5).

TABLE 1

INCORPORATION OF ADENINE-8-C^{14} INTO RNA ADENINE (A) AND GUANINE (G)

	Relative specific activities									
	Intestines		Liver		Kidney		Spleen		Tumor	
	A	G	A	G	A	G	A	G	A	G
Control rat	.93	.14	.79	.15	.69	.17	.82	.11		
Tumor-bearing rat	.93	.14	1.07	.20	1.04	.36	1.01	.16	.69	.081

TABLE 2

INCORPORATION OF 2,6-DIAMINOPURINE INTO PNA GUANINE

	Relative specific activities				
	Intestines	Liver	Kidney	Spleen	Tumor
Control rat	.46	.48	.79	.68	
Tumor-bearing rat	.43	.46	.84	.48	.22

TABLE 3

INCORPORATION OF GUANINE-8-C^{14} INTO PNA GUANINE

	Relative specific activities				
	Intestines	Liver	Kidney	Spleen	Tumor
Control rat	.013	.017	.007	.018	
Tumor-bearing rat	.024	.024	.008	.036	<.001*

* 1.6 μM of the tumor guanine contained no counts above background. An RSA of 0.001 would have resulted in 2 counts/minute above background.

Glycine.—The determination of the synthesis of purines from glycine serves as a measure of the synthesis *de novo* of these compounds. The RSA values are proportional to the amount of synthesis *de novo*, but not equal to it, since the administered glycine is diluted by endogenous pools.[2] These data (Table 5) show that the extent of synthesis *de novo* of tumor PNA is quite high and comparable to that of intestines. The PNA purines of the spleen are synthesized *de novo* to a considerable extent as is indicated by the large amount of incorporation of glycine. There is a striking increase in the amount of glycine incorporated into the PNA of every tissue measured except the intestines in the tumor-bearing animals relative to the controls. The same effect was noted with hamsters (5).

DISCUSSION

The incorporations of the various precursors into the different tissues of the rat and of the hamster lend themselves to many and various comparisons; three deductions are most noteworthy: First, species differences between the rat and the hamster which manifest themselves in the relative utilizations of various precursors and in differences in the relative metabolic activities of different organs in the two animals. Second, there is evidence that tumors affect the host's RNA metabolism. Finally, tumors evidence a metabolic character that is maintained in different heterologous hosts.

Certain shifts in the host's metabolism that have been observed with the rat have also been reported with the hamster with HS #1 and HEP #3 and, therefore, appear to be true manifestations of tumor-host interaction. The increased utilization of adenine by the liver is one case in point. This effect has been found in hamsters with both tumors, HS #1 and HEP #3 (5), and in mice with homologous tumors (4, 6). Although small, the consistency with which this difference is observed makes the effect appear significant. The influence of the tumor on the kidney was found with HS #1 in the hamster but not with the carcinoma. With the HS #1 or HEP #3 present there was an increased utilization of exogenous hypoxanthine for the synthesis of the PNA of liver and intestines. The same results were obtained with the tumor-bearing rats. When glycine was administered to tumor-bearing animals, there was a general increase in incorporation into the PNA compared with that observed with the control animals. This same observation was made in both the hamsters and the rats.

These studies have led, as have those with the hamster, to the demonstration that tumors can evidence an anabolic pattern for PNA which is distinguishable from that of the host's tissues. They incorporate exogenously supplied guanine and hypoxanthine into the PNA to an extremely small extent, and, furthermore, they do not use any preformed purine extensively. An outstanding characteristic of tumors is the preference for purines which have arisen by synthesis *de novo*. This also appears to be a characteristic of other rapidly growing tissues, e.g., the intestines. There is a related observation that neither tumors nor fetuses were able to utilize the purine catabolites of labeled hosts (8), despite the fact that in both cases considerable nucleic acid synthesis was occurring. Too little is known concerning the metabolic routes involved in the conversion of exogenous purines and small molecules into the purine entity of PNA to permit a full evaluation of the pattern. It is also impossible to say whether this dependence on purines arising by synthesis *de novo* is a result of rapid or neoplastic growth or whether the greater growth is a consequence of a shifted balance of alternate metabolic pathways. The question of the extent to which tumor tissues adapt to

TABLE 4

INCORPORATION OF HYPOXANTHINE-8-C^{14} INTO PNA ADENINE (A) AND GUANINE (G)

	Relative specific activities									
	Intestines		Liver		Kidney		Spleen		Tumor	
	A	G	A	G	A	G	A	G	A	G
Control rat	.053	.041	.046	.040	.072	.042	.027	.019		
Tumor-bearing rat	.090	.050	.090	.081	.057	.045	.039	.030	.032	.027

TABLE 5

THE SYNTHESIS OF PNA ADENINE (A) AND GUANINE (G) FROM GLYCINE-1-C^{14}

	Relative specific activities									
	Intestines		Liver		Kidney		Spleen		Tumor	
	A	G	A	G	A	G	A	G	A	G
Control rat	.080	.077	.0040	.0041	.0063	.0038	.040	.033		
Tumor-bearing rat	.081	.063	.0099	.0092	.0111	.0097	.061	.062	.057	.058

[2] Arnstein and Neuberger (1) have shown that the instantly available glycine pool in the rat (for hippuric acid synthesis) is 100 mg/kg. This means that at an absolute minimum the glycine in these experiments was diluted almost twelvefold.

the general pattern of metabolism manifested by the host cannot be fully answered, but it is possible to partially evaluate their degree of metabolic autonomy from the present data.

If a system such as has been studied here is analyzed, i.e., rats and hamsters, with and without tumors, five relationships between tumor and the two hosts are possible. The two hosts can incorporate a given precursor to the same or different extents; in both situations the tumor can use the particular precursor in the second host to the same or greater or lesser extent, e.g.,

Hosts	Tumor	Type
Uptake same in host A and B:	Uptake same in both hosts	1
	Uptake greater in A or B	2
Uptake greater in A than B:	Uptake greater in A than B	3
	Uptake same in A as B	4
	Uptake greater in B than A	5

From the results obtained, we find that diaminopurine, which is used by both hosts to the same extent, is used equally well by the tumor in both hosts. The other precursors are used better in one host than in the other, and each possible level of utilization by the tumor has been observed. The uptake of guanine is an example of relationship type 3; hypoxanthine, type 4; and adenine, type 5. These facts can be viewed as support for the hypothesis that the tumor retains its own individuality of pattern and does not adapt itself to that of the host within the limits of its dependence on the host's transport facilities to bring metabolites to it.[3]

That, under the varied conditions of implantation in two different hosts, the tumor exhibits those consistencies which it does is striking and affords evidence that tumors possess definite characteristic anabolic traits. From the point of view of possible chemotherapy of cancer this is encouraging, since it would indicate that tumors are tissues metabolically independent of the host, and therefore differential susceptibility to antimetabolites can be anticipated.

SUMMARY

The incorporation of several C^{14}-labeled purine precursors into the PNA of rats carrying a human tumor, HS #1 (Toolan), has been determined. The utilization by the tumor, intestine, liver, kidney, and spleen has been measured. Comparison of the values with those previously obtained with hamsters carrying human tumors indicates that certain effects of tumors on host metabolism are independent of the species. These are (*a*) increased utilization of adenine by the liver, (*b*) increased utilization of hypoxanthine for synthesis of PNA of liver and intestine, and (*c*) a general increase in glycine uptake.

The results support the concept that tumors preferentially synthesize nucleic acid purines *de novo* rather than utilize exogenously supplied purines. The tumors appear to possess metabolic characteristics distinguishable from those of the host.

[3] Substances administered parenterally might be chemically changed or destroyed before they reach the tumor. It is obvious, therefore, that *in vivo* incorporation studies cannot demonstrate the ability of the tumor to utilize such potential metabolites.

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